Opposite Effects of Prolactin and Corticosterone on the Expression and Activity of 3β-Hydroxysteroid Dehydrogenase/Δ^5-Δ^4 Isomerase in Rat Skin

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In rat skin, type IV is the major 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD) isoenzyme expressed. Although types I and II 3β-HSD mRNAs are also present in the skin, their level of expression is about two orders of magnitude lower than that of type IV. In this study, we have investigated the control of type IV 3β-HSD mRNA levels as well as 3β-HSD enzymatic activity in hypophysectomized adult rats of both sexes. Skin 3β-HSD activity was measured by the conversion of [^{14}C]-dehydroepiandrosterone into [^{14}C]-androstenedione, whereas ribonuclease protection assay using a specific type IV cRNA probe was used to assess mRNA levels. Intact male and female rats show a similar level of skin 3β-HSD activity, although hypophysectomy caused opposite effects, a decrease being observed in males while an increase was observed in hypophysectomized female animals. We next studied the effects of hyperprolactinemia, corticosterone and l-thyroxine in hypophysectomized animals. L-thyroxine was found to stimulate 3β-HSD expression and activity in male rats whereas no significant effect was observed on the already elevated levels in hypophysectomized female rats. Corticosterone caused an inhibition of type IV 3β-HSD mRNA levels and activity in both male and female animals. Hyperprolactinemia achieved by pituitary implants inserted under the kidney capsule stimulated the expression of type IV mRNA as well as 3β-HSD enzymatic activity in hypophysectomized male and female animals. The present data demonstrate the multihormonal regulation of 3β-HSD/isomerase expression and activity in the rat skin.
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The conversion of Δ^5-3β-hydroxysteroid precursors into the corresponding Δ^4-ketosteroids by isoenzymes of the 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD) family is an obligatory step in the biosynthesis of all classes of hormonal steroids [1]. The expression and/or activity of 3β-HSD subtypes has been described in all classical steroidogenic tissues, namely the adrenal cortex, testis, ovary, and placenta, as well as in numerous peripheral target tissues including the skin, liver, fat, kidney, uterus, epididymis, mammary gland, and prostate in the human and other mammalian species [1–9]. It is becoming recognized that 3β-HSD in peripheral tissues plays an important role in local steroid biosynthesis where it catalyses the transformation of dehydroepiandrosterone (DHEA) into active sex steroids, thus providing steroids that exert their action in the same cells which are responsible for their synthesis, a new area called intracrinology [10,11].

Although a series of studies have addressed the role of sex steroids in the control of hair growth and sebaceous gland physiology, the importance of rat skin as a site of regulated steroid biosynthesis and metabolism has received little attention. Rat hair growth and sebaceous gland activity have been shown to be modulated by pituitary hormones, namely growth hormone (GH) [12], prolactin (PRL) [13], adrenocorticotropic hormone (ACTH) via formation of corticosteroids [14,15], and thyroid-stimulating hormone (TSH) via thyroid hormones [16] as well as melanocyte-stimulating hormone (MSH) ([17], see [18] for review). The presence of 3β-HSD in rat skin has been reported [19,20] and local rat skin steroidogenesis has also been suggested to modulate sebaceous gland physiology [21]. Following these early pioneering studies, little attention has been given to this subject, which was possibly awaiting the availability of the molecular biology tools recently made available [4,5].

So far, in the rat, the structure of four 3β-HSD isoenzymes has been described and the expression of their corresponding mRNAs has been characterized in many tissues [4,5,22–25]. The rat types I and II 3β-HSD expressed in the adrenals, gonads, kidneys, placenta, and adipose tissue share 93.8% homology. The type I 3β-HSD protein has a relative specificity much higher than that of the type II protein. On the other hand, the liver-specific type III protein, sharing approximately 80% homology with the other rat isoenzymes, does not display the expected classical 3β-HSD activity but is rather a 3-ketosteroid reductase, which catalyzes the conversion of 3-keto saturated steroids such as DHT into their corresponding 3β-hydroxy metabolite. Finally, the rat type IV 3β-HSD shares 90.9%, 87.9%, and 78.8% identity with that of rat types I, II, and III 3β-HSD proteins, respectively. The affinity and the specific activity of rat type IV are similar to those of the rat type I 3β-HSD isoenzyme.

In the skin, type IV 3β-HSD is the major mRNA species expressed at levels at least two orders of magnitude higher than that of the type I and type II isoenzymes [26]. The highly predominant expression of the type IV 3β-HSD gene in the skin offers a unique opportunity to investigate the hormonal control of this newly described gene and to further improve our knowledge on the control of skin steroidogenesis. The present study investigates the effect of l-thyroxine or corticosterone as well as hyperprolactinemia on rat

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skin type IV 3β-HSD mRNA levels as well as on 3β-HSD enzymatic activity.

MATERIALS AND METHODS

Animals Intact or hypophysectomized adult male and female Sprague-Dawley rats (Crl : CD(SD)Br) were purchased from Charles River (St-Constant, Québec, Canada) and housed two per cage in a light (14-h light/d; lights on at 0600 h) and temperature-controlled (22 ± 1 C) environment. The intact rats received Agway ProLab R-M-H diet 4018 (ProLab) and water *ad libitum*, whereas hypophysectomized animals received, in addition, oranges, apples, and 5% glucose in 0.9% NaCl.

Hormones Corticosterone (CORT) was purchased from Steraloids (Wilton, NH) and administered subcutaneously twice daily in 5% ethanol, 0.9% NaCl, 1% gelatin, and water (vehicle). L-thyroxine (T4) was from Sigma (St. Louis, MO) and administered in a 10^{-3} N NaOH, 0.9% NaCl, 1% gelatin-H_2O (pH 10.5).

Experimental Procedures Male and female animals were assigned randomly to the following groups: a) Intact rats with 1) vehicle alone; and b) hypophysectomized rats with 2) vehicle alone, 3) vehicle plus pituitary implants (PI), 4) corticosterone (350 μg), and 5) l-thyroxine (T4) (25 μg). In the appropriate groups, three anterior pituitaries removed from intact female rats (body weight 300 to 400 g) were inserted under the left kidney capsule 2 d before starting the experiment.

Hormone Measurements Serum PRL was measured by double-antibody RIA using rat PRL-I-5 for iodination, rat PRL-RP-3 as reference preparation, and rabbit antiserum (anti-rPRL-S-9), kindly supplied by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Rat PRL-RP-3 contains 1% bovine serum albumin.

RNA Isolation and Hybridization Analyses Total RNA was extracted from shaved dorsal skin with an automated nucleic acid extractor (Model 340, Applied Biosystems Inc. Foster City, CA) according to supplier's protocol. Total RNA thus obtained was solubilized in sterile water, ethanol-precipitated, and resuspended in sterile water. Poly A^+ RNA was purified using oligo-dT cellulose chromatography [27]. Samples of rat skin polyA^+ RNA (4 μg) were hybridized to a specific type IV cRNA probe (284 bp) [26] and then digested with ribonucleases A and T1. The protected fragments (157 bp) were resolved on a denaturing polyacrylamide-urea sequencing gel as previously described [4,5]. Signals were detected and quantified with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Results are expressed as mean ± SEM (n = 3–4) in arbitrary units (control groups were set at 100).

Enzymatic Assay of 3β-HSD Activity Assays of 3β-HSD activity were performed as previously described [28–31]. Briefly, pieces of dorsal skin (about 1 g) were homogenized in 2 ml of phosphate buffer (pH 7.5) containing protease inhibitors and centrifuged for 30 min at 1000 × *g*. One hundred microliters of the supernatant were then incubated for 45 min at 37°C in a total volume of 0.5 ml phosphate buffer containing 0.75 μM [^{14}C]-dehydroepiandrosterone (DHEA) and 0.8 mM NAD^+. Labeled DHEA (51 mCi/mmol) was from DuPont (Markham, Canada) and purified by thin-layer chromatography (TLC) before use as previously described [29]. The enzymatic reaction was stopped by chilling the incubation mixture in an ice-water slurry and adding 3 ml of diethyl ether and mixing. The components were then frozen in a dry ice-ethanol bath. The liquid organic phase was kept while the frozen aqueous fraction was reextracted once with ether. The organic phases were then pooled and evaporated to dryness under a nitrogen stream. The [^{14}C]-Δ^4-dione produced from [^{14}C]-DHEA was then separated on TLC before autoradiography for 2 d. The TLC areas corresponding to DHEA and Δ^4-dione were cut and transferred into vials containing 0.5 ml ethanol and 10 ml of scintillation liquid was added. The radioactivity was then measured. Protein content was measured by the method of Bradford [32]. Results are expressed as means ± SEM (n = 9) in pmoles of androstenedione (Δ^4-dione) formed/mg protein/min.

Statistical Analysis Statistical significance was determined according to the multiple-range test of Duncan-Kramer [33].

RESULTS

The level of rat skin 3β-HSD activity measured by the conversion of [^{14}C]-DHEA into [^{14}C]-Δ^4-dione is comparable in male and female intact animals (4.71 ± 0.53 pmoles of [^{14}C]-Δ^4-dione formed/mg protein/min versus 4.04 ± 0.38 pmoles). Hypophysectomy, however, had opposite effects on this parameter in animals of the two sexes: thus, although in male rats hypophysectomy caused a 34% ($p < 0.01$) decrease in 3β-HSD activity, it induced a 40% stimulation ($p < 0.05$) of the same parameter in female rats.

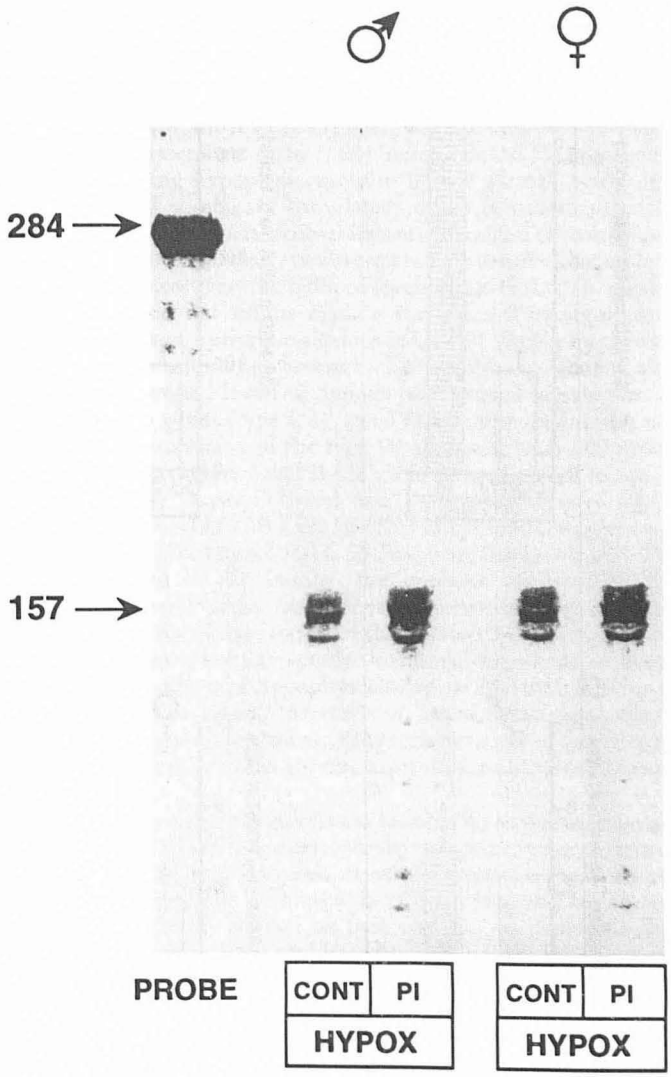

Figure 1. Effect of prolactin on 3β-HSD expression. Ribonuclease protection analysis of the effect of hyperprolactinemia achieved by pituitary grafts (PI) under the kidney capsule on type IV 3β-HSD mRNA levels in the skin of hypophysectomized male and female rats. Control animals (CONT) and animals bearing three pituitary grafts each received the vehicle for 9 d starting 15 d after surgery. Samples of rat skin poly A^+ RNA (4 μg) were hybridized to a specific type IV cRNA probe (284 bp) and then digested with ribonucleases as described in *Materials and Methods*. The protected fragments (157 bp) were resolved on a denaturing polyacrylamide-urea sequencing gel as previously described [4,5]. Signals were detected with a phosphorImager.

As shown in Fig 1, with 4 μg of male hypophysectomized rat skin poly A^+ RNA the expected protected fragment of 157 nucleotides is detected after ribonuclease digestion, and hyperprolactinemia achieved by pituitary implants (PI) caused a marked increase in type IV mRNA levels. Similarly, in female skin, PI increased type IV 3β-HSD mRNA levels. Signals of the 157 nucleotides-protected fragment were quantified, and results are represented in Fig 2*A* in arbitrary units. It can be seen that pituitary implants had a stimulatory effect on type IV 3β-HSD mRNA levels in hypophysectomized male skin as well as female skin. In male rats, mRNA levels were increased by 3.4 times ($p < 0.01$) over hypox controls, whereas in female rats the same treatment caused a 2-times ($p < 0.01$) increase. Pituitary implants (PI) grafted under the kidney capsule raised serum PRL in hypophysectomized rats to levels about 4.5 (female) and 7 (male) times higher than those measured in intact

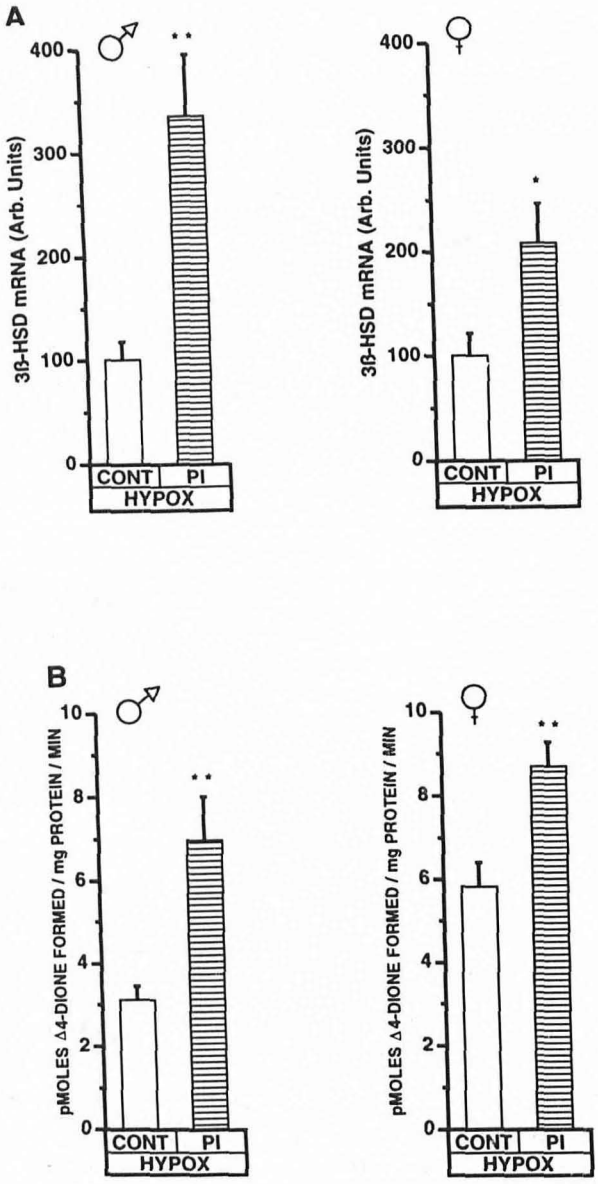

Figure 2. Effect of prolactin on 3β-HSD activity. Effect of hyperprolactinemia achieved by pituitary grafts (PI) on 3β-HSD mRNA levels *(A)* and enzymatic activity *(B)* in hypophysectomized male and female rats. Animals were treated as described in the legend to Fig 1. Signals corresponding to the 157 nucleotide protected fragments were quantified with a phosphorImager and 3β-mRNA levels are expressed in arbitrary units (control groups set at 100) as means ± SEM (n = 3–4). Skin homogenates were incubated with 0.75 μM of [^{14}C]-DHEA and 0.8 mM NAD$^+$ as described in *Materials and Methods*. The data are presented as means ± SEM of nine independent derterminations of skin 3β-HSD activity expressed in pmoles of Δ^4-dione formed/mg protein/min. **$p < 0.01$ versus control group.

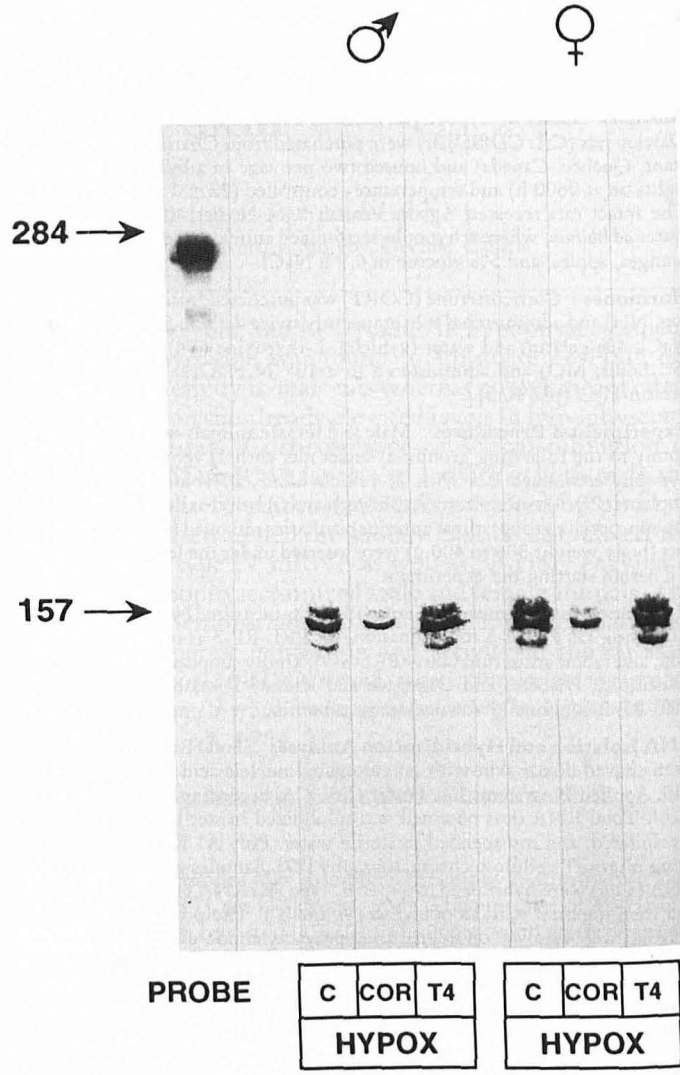

Figure 3. Effect of glucocorticoid and thyroid hormones on 3β-HSD expression. Ribonuclease protection analysis of the effect of treatment with corticosterone (COR) or l-thyroxine (T4) on type IV 3β-HSD mRNA levels in the skin of hypophysectomized male and female rats. Corticosterone (COR, 350 μg) and T4 (25 μg) were administered twice daily for a period of 9 d starting 15 d after hypophysectomy. Hypophysectomized control animals (C) received the vehicle alone for 9 d. Samples were processed as described in legend to Fig 1.

control animals (data not shown). In addition to the changes in 3β-HSD mRNA levels, hyperprolactinemia caused a highly significant stimulation of 3β-HSD activity in both male and female hypophysectomized animals (Fig 2*B*). In fact, in the male hypohysectomized rat, skin 3β-HSD enzymatic activity was increased to levels 1.2 times ($p < 0.01$) above those of hypox animals, whereas in the female the levels achieved were 50% above those achieved by hypophysectomy ($p < 0.01$).

We next investigated the effect of treatment with CORT and T4 on the same parameters in hypophysectomized animals. As observed in Fig 3, CORT (350 μg, twice daily) clearly inhibited type IV 3β-HSD mRNA levels in both male and female hypox animals, whereas T4 (25 μg, twice daily) stimulated this parameter only in male rats. In fact, as shown in Fig 4*A*, CORT decreased type IV 3β-HSD mRNA levels by 31% ($p < 0.05$) in male and 53% ($p < 0.01$) in female rats compared to the corresponding hypox control groups.

On the other hand, T4 stimulated 3β-HSD mRNA levels in male animals 50% over hypox controls ($p < 0.05$), whereas no effect was observed in female animals. Similarly, as shown in Fig 4*B*, T4 caused a 60% increase of skin 3β-HSD activity in hypox male animals, whereas no effect was observed in female animals. Corticosterone (350 μg, twice daily) caused a 38% decrease ($p < 0.01$) in female-skin 3β-HSD activity, whereas at this dose no effect was observed in male animals. However, at a higher dose (1.5 mg), CORT decreased significantly male-skin 3β-HSD activity to levels observed in female animals (data not shown).

DISCUSSION

Our data show clearly that the type IV 3β-HSD gene specifically expressed in rat skin is regulated by the pituitary hormone PRL as well as by thyroid and glucocorticoid hormones. Considering that

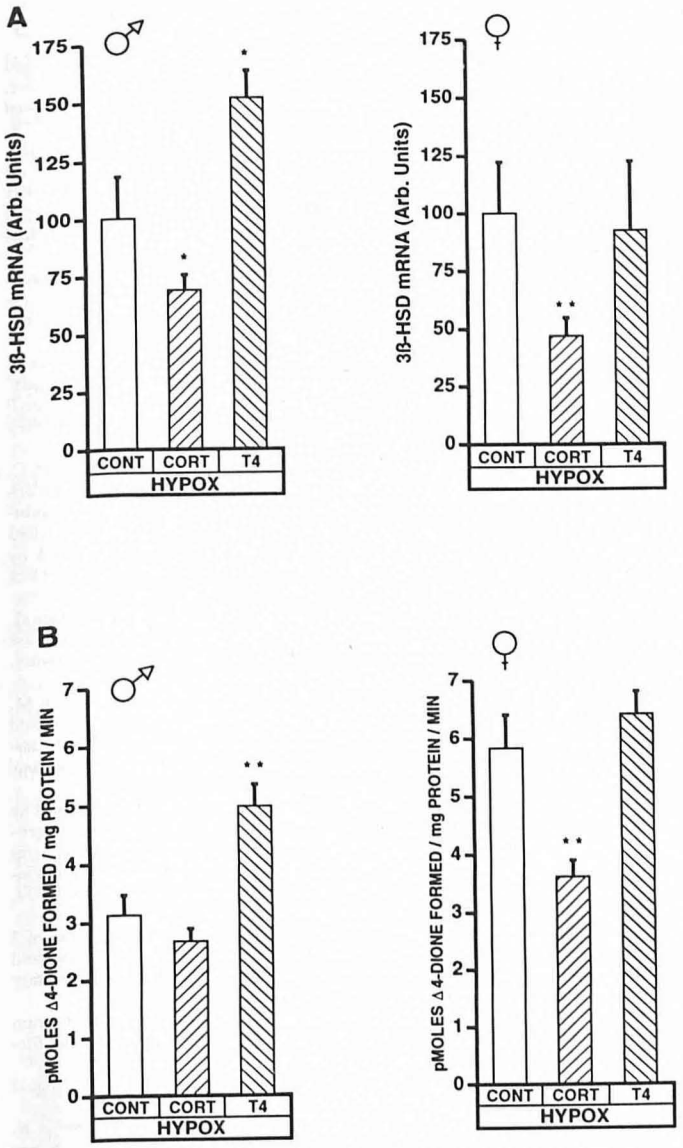

Figure 4. Effect of glucocorticoid and thyroid hormones on 3β-HSD activity. Effect of corticosterone (CORT) or thyroxine (T4) on 3β-HSD mRNA levels *(A)* and enzymatic activity *(B)* in the skin of hypophysectomized male and female rats. Animals were treated as described in the legend to Fig 3 and samples processed as described in legend to Fig 2. The mRNA levels are expressed in arbitrary units (control groups set at 100) as means ± SEM of 3–4 independent determinations. Three β-HSD activity is presented in pmoles of Δ^4-dione formed mg protein/min as means ± SEM of nine independent determinations.*$p < 0.05$, **$p < 0.01$ versus hypox control group.

the skin is the largest organ in the body, 3β-HSD could play an important role, not only in skin physiology, but also in the global steroid formation of the whole animal. Interestingly, sexual dimorphism was observed for skin 3β-HSD in response to various hormonal manipulations. Pituitary removal stimulates female skin 3β-HSD activity, whereas it inhibits the same parameter in male animals. This phenomenon could be partly explained by the observation that androgens stimulate 3β-HSD activity in male rat skin, whereas estrogens inhibit this parameter in females (unpublished data). Thus, following hypophysectomy, the sex-specific decrease in steroid formation by the gonads could modulate skin 3β-HSD activity. In addition, T4 stimulates skin 3β-HSD activity and expression only in male hypohysectomized animals, whereas CORT, at physiologic doses, shows inhibitory effects on skin activity only in female rats. The levels of 3β-HSD enzymatic activity were, however, reduced by high doses of CORT in animals of both sexes (data not shown). On the other hand, hyperprolactinemia stimulated both male and female skin 3β-HSD type IV mRNA levels and activity.

The most significant sex dimorphism was the one observed following hypophysectomy. In fact, the increase in 3β-HSD activity observed following hypophysectomy in female animals could explain the lack of significant stimulatory effect of treatment with thyroid hormones in female animals because maximal or near-maximal levels of 3β-HSD activity could have been reached by hypophysectomy alone. However, the reduced levels of 3β-HSD in hypophysectomized male rats might explain the absence of significant inhibitory effects of a physiologic dose of CORT [34] in hypophysectomized male animals, whereas a clear inhibitory effect is observed on the elevated levels of hypophysectomized female rats.

Three β-HSD genes (type I, II, and IV) are expressed in the rat skin, although expression of the type IV gene is at least 100 times higher than that of types I and II [26]. We have observed in other studies (Martel *et al,* unpublished data) opposite effects of PRL, thyroid hormone, and CORT on 3β-HSD enzymatic activity in rat skin and kidney. Since type I and II 3β-HSDs are the genes predominantly expressed in the kidney, the opposite effects of PRL, CORT, and thyroid hormones in these two tissues could indicate not only specificity of expression of the various 3β-HSD genes in the skin and kidney, but also specific hormonal regulation in these two tissues. The effect of hypophysectomy on 3β-HSD activity is also different in the kidney (Martel *et al,* unpublished data). Such observations suggest differences in the promoter areas of the various 3β-HSD genes and/or tissue-specific expression and activity of regulatory proteins.

L-thyroxine reversed the inhibition induced by hypophysectomy on male skin 3β-HSD/isomerase activity and expression, whereas female skin 3β-HSD activity and expression remained unchanged after the same treatment. Although the effects of thyroid hormones have been extensively studied on hair growth, no data were yet available on the effect of thyroid hormones on rat skin steroidogenesis.

Hyperprolactinemia achieved by pituitary grafts implanted under the kidney capsule succeeded in stimulating female skin 3β-HSD activity and type IV mRNA expression but to a lower extent than in male animals. It is interesting to observe the relatively low sensitivity of female skin 3β-HSD to prolactin compared to that of male animals. Similar conclusions were reached for the effect of prolactin in the female kidney although an opposite effect was observed (Martel *et al*, unpublished data).

Corticosterone at the physiologic dose used (350 μg, twice daily) [34] decreased skin 3β-HSD in female hypophysectomized rats but not male. When a higher dose (1.5 mg, twice daily) was given to hypox male animals, comparable inhibitory effects were observed in the male and female animals. It is of interest to observe that the opposite situation compared to prolactin treatment is found in response to corticosterone. In fact, male skin 3β-HSD activity was less responsive to the glucocorticoid than that of female skin.

In summary, our data demonstrate that skin 3β-HSD is regulated by different hormones. Whereas hypophysectomy caused opposite effects, the effect being stimulatory on skin 3β-HSD activity in female and inhibitory in male rats, T4 and prolactin had stimulatory effects, and corticosterone was inhibitory. Such data suggest that the skin might well be an important site of steroid formation under stringent control by pituitary and sex hormones.

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INTERNATIONAL RESEARCH WORKSHOP ON ALOPECIA AREATA

The Second International Research Workshop on Alopecia Areata will be held on November 7 and 8, 1994. The two-day workshop will bring together investigators for an open exchange of recent advances in alopecia areata, as well as studies of hair follicle biology. Topics will include immunogenetics, target tissues, dermal-epidermal instructions, stem cells, growth factors, and animal models. Results of this workshop will guide further research in this field.

The workshop is co-sponsored by the National Alopecia Areata Foundation (NAAF) and the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) and will be held at the Lister Hill Auditorium on the campus of the National Institutes of Health (NIH) in Bethesda, MD. For more information call the NAAF office at (415) 456-4644.